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Indirect Human Health Risk Assessment of the GloFish® Cosmic Blue® (BS2017), GloFish® Electric Green® (GS2017), GloFish® Sunburst Orange® (OS2016), and the GloFish® Galactic Purple® (PS2016) Sharks (*Epalzeorhynchos frenatum*), for use as Ornamental Aquarium Fish in Canada

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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TABLE OF CONTENTS

ABSTRACT iv
INTRODUCTION 1
HAZARD ASSESSMENT 1
IDENTIFICATION AND CHARACTERIZATION OF *EPALZEORHYNCHOS FRENATUM* BS2017, GS2017, OS2016, AND PS2016 1
Binomial name 1
Taxonomy 1
Synonyms, common and superseded names 2
Characterization and substantiation of the taxonomic identification 2
Strain History 2
Genetic modifications: purpose, method, genetic and phenotypic changes 3
Biological and ecological properties 3
HUMAN HEALTH EFFECTS 3
Zoonotic potential 3
Allergenicity/Toxigenicity 7
History of Use 8
HAZARD CHARACTERIZATION 8
UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH HAZARD ASSESSMENT 9
EXPOSURE ASSESSMENT 10
INTENDED USE 10
Import and distribution to retailers in Canada 10
Introduction in Canada as ornamental fish in home aquaria 11
OTHER POTENTIAL USES 12
Fish uses for the general population 12
Scientific research 12
Manufacture in Canada 13
Conclusion on potential uses for general population 13
ENVIRONMENTAL RELEASES AND FATE IN THE ENVIRONMENT 13
Releases of live fish into water bodies 13
Release of live or dead fish on land or dead fish into water bodies 14
EXPOSURE CHARACTERIZATION 15
UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT 15
RISK CHARACTERIZATION 17
NOTIFIED USE 17
OTHER POTENTIAL USES 17
RISK ASSESSMENT CONCLUSION 17
REFERENCES CITED 18

ABSTRACT

An indirect human health risk assessment was conducted on four lines of genetically modified Rainbow Sharks (*Epalzeorhynchos frenatum*) known as the GloFish® Cosmic Blue® Shark (BS2017), GloFish® Electric Green® Shark (GS2017), GloFish® Sunburst Orange® Shark (OS2016), and the GloFish® Galactic Purple® Shark (PS2016), that were notified under the *Canadian Environmental Protection Act* (CEPA). BS2017, GS2017, OS2016, and PS2016 are modified lines of diploid, hemizygous or homozygous, Rainbow Sharks, containing genes encoding for different fluorescent proteins and a chromoprotein (BS2017 only). BS2017, GS2017, OS2016, and PS2016 appear blue, green, orange, or purple, respectively under ambient light (including sunlight). The four lines will be imported from the United States for use as ornamental fish in home aquaria. This risk assessment examined the potential for the four lines to cause harmful effects to humans in Canada relative to wild-type Rainbow Sharks as a consequence of environmental exposure including under its intended use in home aquaria. The parental strain, *E. frenatum*, has been available as a home aquarium fish since the 1970s without any reported adverse human health effects. There is no evidence to suggest a risk of adverse human health effects for the general Canadian population from use of BS2017, GS2017, OS2016, and PS2016 as ornamental aquarium fish as well as other potential uses. As such, there is no expectation that BS2017, GS2017, OS2016, and PS2016 will pose any more risks to human health than wild-type *E. frenatum*.

INTRODUCTION

The following indirect human health risk assessment was conducted on *Epalzeorhynchos frenatum* BS2017, GS2017, OS2016, and PS2016, four genetically modified lines of diploid, hemizygous or homozygous, Rainbow Sharks containing genes coding for recombinant fluorescent blue, green, yellow, or purple proteins, respectively. BS2017 also contains a gene for a blue chromoprotein. BS2017, GS2017, OS2016, and PS2016 are blue, green, orange, and purple in colour, respectively, when displayed in ambient light, including sunlight. This risk assessment examines the potential for the importation of BS2017, GS2017, OS2016, and PS2016 from the United States to cause harmful effects to humans in Canada, as a consequence of exposure in natural environments and environments under the intended use as ornamental fish in home aquaria. This risk assessment was conducted relative to the wild-type *E. frenatum,* which have widespread use in Canada and other parts of the world as tropical ornamental fish. The risk assessment was conducted under the *Canadian Environmental Protection Act* (CEPA) and *New Substances Notification Regulations (Organisms)*.

HAZARD ASSESSMENT

IDENTIFICATION AND CHARACTERIZATION OF *EPALZEORHYNCHOS FRENATUM* BS2017, GS2017, OS2016, AND PS2016

Binomial name

Epalzeorhynchos frenatum (Fowler 1934) BS2017, GS2017, OS2016, and PS2016

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Actinopterygii
Class	Teleostei
Order	Cypriniformes
Family	Cyprinidae
Genus	*Epalzeorhynchos*
Species	*Frenatum*
Strains	BS2017, GS2017, OS2016, and PS2016

Synonyms, common and superseded names

Synonym/common names: *Labeo frenatus*, *L. erythrurus*, *E. frenatus,* Rainbow Shark, Ruby Shark, Red-fin Shark, White-fin Shark, Rainbow Shark Minnow

Trade names:

- BS2017 - GloFish® Cosmic Blue® Shark
- GS2017 - GloFish® Electric Green® Shark
- OS2016 - GloFish® Sunburst Orange® Shark
- PS2016 - GloFish® Galactic Purple® Shark

Characterization and substantiation of the taxonomic identification

Epalzeorhynchos frenatum BS2017, GS2017, OS2016, and PS2016 are genetically modified lines of diploid, hemizygous or homozygous, Rainbow Sharks containing genetic constructs which makes them appear blue (BS2017), green (GS2017), orange (OS2016), or purple (PS2016) under ambient light, including sunlight. All four lines were derived from a domestic albino strain of Rainbow Shark.

Rainbow sharks are named for their shark-like elongated bodies as well as the appearance of their dorsal fin (Woods 2022; Yang 2022). Their colouration may be gray, black, or dark blue with brightly coloured red or orange fins (Woods, 2022; Leighton 2023). Bodies consist of a pointed snout, elongated head, large eyes with a darkened iris, a well-developed mouth with barbels, flat abdomen and sharp tail ends connecting with the tail (Woods 2022; Leighton 2023). They also possess a conspicuous black band extending from the snout to the eye and a dark spot near the tail (Aqua-Fish 2014; Seriously Fish 2023). The albino variant possesses the same fin colouration with bodies ranging from white with pinkish undertones to pale orange or yellow and red eyes and lacks the dark spots (Aqua-Fish 2014; Edmond 2022; Woods 2022; Leighton 2023). It is difficult to determine sex until maturity where females will be rounder and males will develop black lines on the tail fin and have a brighter and more vivid colouration (Edmond 2022).

Rainbow Sharks can be easily distinguished from the similar looking Red-Tail Shark (*Epalzeorhynchos bicolor*) as the former species lacks the intense ebony colour as well as from the black colouration of the pectoral, dorsal, and anal fins in the latter species (Purser 2020; Herzog 2023). Also similar in appearance is the Red Fin Shark (*Epalzeorhynchos munense*) which is distinguishable from the black margins on the pelvic and pectoral fins as well as from the position of the dorsal and pelvic fins (Tropical Fish Keeping 2016).

Strain History

The notified lines BS2017, GS2017, OS2016, and PS2016 were produced by microinjection of the expression cassettes containing the corresponding transgenes into blastomeres of *E. frenatum* eggs. Greater detail regarding strain development and history of the notified lines has been provided by the company for the expressed purpose of the current risk assessment and review, but is identified as confidential business information and is not included in this report. Broodstocks for BS2017, GS2017, OS2016, and PS2016 are maintained separately with the same breeding protocol used for all four lines. Furthermore, to maintain line integrity, non-transgenic fish produced during development of the lines were humanely euthanized and disposed of according to the notifier’s protocols.

Genetic modifications: purpose, method, genetic and phenotypic changes

The notified lines which have been modified to appear blue (BS2017), green (GS2017), orange (OS2016), or purple (PS2016) under ambient light, including sunlight, are intended for use by the general public for home aquarium display purposes only. Just like the wild-type *E. frenatum*, which is a non-food species that has been used safely in aquaria worldwide since the 1970s (Brand 2020), BS2017, GS2017, OS2016, and PS2016 are not intended for food use.

According to the information provided by the notifier, in addition to BS2017, GS2017, OS2016, and PS2016 appearing blue, green, orange, and purple, respectively, under ambient light, BS2017, GS2017 and PS2016 were sensitive ($P \leq 0.05$) to low water temperatures compared to their non-transgenic siblings. There was no significant difference ($P > 0.05$) in temperature sensitivity between OS2016 fish and their non-transgenic siblings.

Biological and ecological properties

Rainbow Sharks are small tropical cyprinid freshwater fish native to Southeast Asia and can be found in rivers in Cambodia, Laos, Malaysia, Myanmar, and Thailand. While preferring rivers with sandy bottoms, Rainbow Sharks are known to migrate into floodplains during periods of heavy rain or flooding. They are known as bottom-feeders and have an omnivorous diet in the wild primarily consisting of algae, periphyton, and small invertebrates (Brand 2020; Yang 2022; Ocean Info 2023). Average life expectancy for Rainbow Sharks is approximately four to six years, but they have been known to live to eight years (Yang 2022).

The maximum length of Rainbow Sharks is approximately 15 cm with the two sexes not being distinguishable by length (Woods 2022). While passive in their natural habitat, they are a territorial and semi-aggressive species when placed in an aquarium (Yang 2022). This territorial and aggressive behaviour is also reported in GloFish® Sharks (Page 2021).

Rainbow Sharks attain sexual maturity when they reach approximately 10 cm in length after one to two years of life (Yang 2022; Leighton 2023). According to the aquarium hobbyist community, breeding of Rainbow Sharks is very challenging with commercial production relying on injecting females with hormones (Edmond 2022; Woods 2022). Reproductive dysfunction is common in captive fish with severity varying by species and administration of exogenous hormones to induce spawning is a typical practice (Sipos et al. 2020). Shireman and Gildea (1989) reported female Rainbow Sharks injected with a combination of carp pituitary extract and human chorionic gonadotropin released approximately 10,000 eggs with 2,000 to 3,000 eggs released during a second spawning. Similarly, Sipos et al. (2020) reported an average of 8544 ± 2789 eggs per spawn in Rainbow Sharks treated with chicken gonadotropin releasing hormone (cGnRH IIa). During spawning, the female will lay her eggs which will be fertilized by a milt spray from the male with the eggs left to develop for approximately a week before hatching (Edmond 2022; Leighton 2023). In nature, breeding typically occurs during the colder seasons around October to November (Edmond 2022; Woods 2022).

HUMAN HEALTH EFFECTS

Zoonotic potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to wild-type *E. frenatum*, or to the other commercially available GloFish® lines. Rainbow Sharks are described in the hobbyist community as being not susceptible to many diseases, but infections are still possible (Edmond 2022). Aquarium pet fish can also carry pathogenic agents, of bacterial, viral, fungal, or parasitic origin, that may have a zoonotic feature endangering the persons handling the animals (Cardoso et al, 2019). There are few reports of zoonoses by parasitic, fungal, and viral pathogens from aquatic organisms with bacteria being reported as the main etiological agents of zoonotic infection (Iqbal et al. 2018). These are largely infections from contact with tropical ornamental fish or through ingestion of food or drinking water that has been contaminated with pathogens and parasites associated with ornamental or aquarium fish.

Contact is the main route of transmission leading to bacterial infections in humans that develop from handling of aquatic organisms (Lowry and Smith 2007). Young children, pregnant women, and immunocompromised individuals are at higher risk for these infections (Dinç et al. 2015). Children are also more susceptible to severe disease outcomes as compared with adults as they often have less stringent hygienic practices (Dunn et al. 2015). While most infections are self-limiting, more serious cases are associated with immune deficiency, infection with highly virulent strains, contact with a large inoculum, depth of skin penetration, or a combination of these factors (Haenen et al. 2020).

Bacterial disease is extremely common in ornamental fish and is most frequently associated with bacteria that are ubiquitous in the aquatic environment acting as opportunistic pathogens secondary to stress (Roberts et al. 2009). Some bacterial species associated with tropical fish capable of causing human illness belong to the genera *Aeromonas*, and *Salmonella* as well as *Mycobacterium marinum,* and *Streptococcus iniae* (CDC 2023) with the most commonly reported infections being associated with *M. marinum* (Weir et al. 2012).

In humans, *M. marinum* is the causative agent for the disease “fish tank granuloma” which results in ulcerative skin lesions or raised granulomatous nodules. These lesions are typically limited to the distal extremities such as the hands, legs, and feet as *M. marinum* has an optimum growth temperature range of 26°C to 32°C (Mutoji and Ennis 2012; Gauthier 2015). However, these nodular cutaneous lesions can progress to tenosynovitis, arthritis and osteomyelitis (Hashish et al. 2018). In addition, rare cases of systemic mycobacteriosis have been reported in immunocompromised individuals (Lowry and Smith 2007). Infections are generally contracted from exposure of wounds and skin abrasions to contaminated water (Gauthier 2015). In humans, mycobacteriosis is classified into four types (I – IV). Type I is found in patients which are immunocompetent with clinical signs including superficial lesions with crusted and ulcerates nodules or verrucuous plaques. The lesions are small, painless, bluish-red papules approximately 1 to 2 cm in diameter. Signs develop over the course of weeks to months. Type II occurs in immunosuppressed individuals and involves lesions with abscesses, inflammatory nodules, and granulomas. The lesions may be single or multiple subcutaneous granulomas, with or without ulceration. In Type III, infections occur in deep tissues with or without skin lesions with clinical signs including arthritis, tenosynovitis, osteomyelitis, and bursitis. Type IV is very rare, but can occur in patients with lung disease (Delghandi et al. 2020). Virulence determinants for *M. marinum* have not been fully elucidated (Narendrakumar et al. 2022).

It is probable that almost all species of fish are susceptible to *Mycobacterium* species with levels of mortality ranging from 10% to 100% of infected fish (Delghandi et al. 2020). *M. marinum*, *M. chelonae*, and *M. fortuitum* are the most commonly reported species to cause piscine mycobacteriosis (Phillips Savage et al. 2022) with *M. marinum* reported in infecting more than 200 species of freshwater, brackish and marine fishes (Narendrakumar et al. 2022). Other examples of species of *Mycobacterium* known to cause infections in fish include *M. abscessus*, *M. flavescens*, *M. gordonae, M. haemophilum*, *M. kansasii*, and *M. peregrinum* (Cardoso et al. 2019; Pate et al. 2019; Puk and Guz 2020). Phillips Savage et al. (2022) reported only one of four Rainbow Sharks collected from four different facilities on Trinidad was presumptive positive for *Mycobacterium* spp.

Although most cases of fish-related infections in humans are caused by *M. marinum*, home aquarists should also be aware of the zoonotic potential of other species of *Mycobacterium* (Puk and Guz 2020). In immunosuppressed humans and children, *M. haemophilum* has been reported to be associated with subcutaneous infections, lymphadenitis, septic arthritis, osteomyelitis, pneumonitis and disseminated disease (Emmerich et al. 2019; Franco-Paredes et al. 2019). Cameselle-Martínez et al. (2007) reported on a cutaneous infection by *M. haemophilum* in a severely immunosuppressed AIDS patient following a bite from an aquarium fish. The infection was successfully treated following a combined therapy of six antibiotics. *M. abscessus*, *M. chelonae*, *M. fortuitum*, and *M. peregrinum* are also associated with cutaneous infections in humans (Kamijo et al. 2012; Franco-Paredes et al. 2019). Li et al. (2014) reported on a successful treatment with antibiotics of a cutaneous *M. chelonae* infection on the left arm of an 82-year-old woman with a hobby of rearing tropical fish. While cutaneaous mycobacterial infections may be successfully resolved with antibiotics, the choice of antibacterial combinations and length of therapy is species-specific (Franco-Paredes et al. 2018). Guz and Puk (2022) examined the antibiotic susceptibility of 99 isolates of nontuberculous mycobacteria (13 species of *Mycobacterium*) from diseased ornamental fish. The authors found the majority of isolates were susceptible to kanamycin, amikacin, clarithromycin, sulfamethoxazole, ciprofloxacin, and doxycycline with most being resistant to isoniazid and rifampicin. An in-house literature search found no reports of human mycobacterial infections attributed to Rainbow Sharks from home aquarium exposure.

Zoonotic infections from *S. iniae* are opportunistic and have most often been associated with puncture wounds from the handling and preparation of infected fish by persons with underlying medical conditions such as diabetes mellitus, chronic rheumatic heart disease, or cirrhosis (Baiano and Barnes, 2009; Haenen et al. 2020, 2023). From the handling of live or recently killed infected fish, *S. iniae* may cause severe disease including septicaemia, endocarditis, arthritis, meningitis, fever, abdominal distension, and pneumonia (Lowry and Smith 2007; Boylan 2011; Gauthier 2015; Haenen et al. 2020). However, the species is referred to as an emerging human pathogen with bacterial cellulitis being the most common clinical disease seen in patients (Glajzner et al. 2021). In addition, human infections by *S. iniae* may be under-reported as identification of this pathogen in clinical laboratories is hampered by limitations of commercial identification systems (Haenen et al. 2023). *S. iniae* is reported to be generally susceptible to antibiotics with therapeutic options in human infections including penicillin, cloxacillin, ampicillin, and cefalexin (Glajzner et al. 2021). Other species capable of causing fish streptococcus include *S. agalactiae*, *S. difficile*, *S. difficilis*, *S. dysgalactiae*, and *S. shiloi* (Ziarati et al. 2022). Rainbow Sharks are one of several ornamental fish species that have been reported to be susceptible to streptococcal infections (Yanong et al. 2007) and *S. iniae* has been isolated from the species (Russo et al. 2006). However, there are no reports in the scientific literature of human streptococcal infections attributed to Rainbow Sharks from home aquarium exposure.

Aeromonas spp. are opportunistic pathogens that are associated with a number of diseases in ornamental fish (Hossain et al. 2018). *Aeromonas hydrophila* is the most commonly reported Aeromonad that possesses zoonotic potential with *A. caviae, A. jandaei, A. sobria, A. salmonidae*, and *A. veronii* also having been reported (Boylan 2011; Zariati et al. 2022). Water with high nutrient levels can cause bacterial blooms capable of being infectious to humans through wounds or ingestion; however, infections are rare and typically involve immune suppression (Boylan 2011). In humans, *A. hydrophila* exposure may result in local skin infections and occasionally, diarrheal disease (Haenen et al. 2020). *A. hydrophila* was one of the species of bacteria isolated from cough swabs of an 11-month-old boy with cystic fibrosis (Cremonesini and Thomson 2008). The authors believe the infection was the result of aerosol spread of the bacterium due to the aeration process of fish tanks in the home as isolations of *A.*

hydrophila only ceased following removal of the tanks. While the report by Cremonesini and Thomson (2008) did not identify the species of fish present in the aquarium, there are no reported cases of *A. hydrophila* zoonotic infections attributed to *E. frenatum* exposure. Among the pathogenic *Aeromonas* spp., *A. veronii* appears to exhibit the broadest host range as species ranging from invertebrates to mammals, including humans, have shown susceptibility to this pathogen (Lazado and Zilberg 2018). *A. veronii* (26.3%) and *A. hydrophilla* (16.2%) were the most commonly isolated bacterial species from 112 fish found positive from a total of 126 fish collected from a wholesaler in São Paulo, Brazil (Cardoso et al. 2021). Aeromonads isolated from human infections have been found to be susceptible to various antibiotics such as fluoroquinolones and third-generation cephalosporins (Haenen et al. 2023). However, an in-house literature search found no reported cases of zoonotic infections of *A. veronii* from ornamental fish exposure.

Salmonella infection can occur through contact with an animal's habitat such as an aquarium (CDC 2023). While *Salmonella* is not a known pathogen for tropical fish, they may act as bacterial reservoirs and excrete *Salmonella* in their feces during periods of stress (Gaulin et al. 2005). Musto et al. (2006) reported on 78 cases of *Salmonella* Paratyphi B biovar Java infections in people having aquaria containing tropical fish in Australia. Infections were mostly seen in children (median age of cases was three years old) following exposure to aquarium water and resulted in diarrhoea, fever, abdominal cramps, vomiting, bloody stool, headaches, and myalgia. Types of tropical fish reported in this study included Tetras, Guppies and Angel Fish. Similarly, out of 53 reported cases of *S.* Paratyphi B, var. Java infections reported in the province of Quebec from January 2000 to June 2003, 33 infected individuals owned an aquarium with 21 of the aquaria testing positive for *Salmonella* (Gaulin et al. 2005). However, the authors did not identify any of the tropical fish species owned by the infected individuals. An in-house literature search found no reports of *Salmonella* zoonotic infections attributed to *E. frenatum* exposure.

Zoonotic infections from fish primarily occur through puncture, cuts, scrapes, abrasions, or sores in the skin (Boylan 2011). Infections may be prevented through wearing gloves when handling fish or cleaning fish tanks and avoiding contact with any potentially contaminated water if any open skin wounds are present. Washing hands and skin with soap and water after contact with aquarium water and fish is also highly recommended. In addition, people with compromised immune systems or underlying medical conditions as well as children should avoid cleaning tanks or handling fish (Haenen et al. 2013; 2020).

There are no reports specifically associating either the notified organisms or wild-type *E. frenatum* with any parasites of human health significance. During line development, hatchery technicians trained to recognize diseases and abnormalities visually examined the fish and any not meeting specific criteria were removed from the population. Routine health evaluations (necropsy, microbiology) were conducted on limited sample sizes of a minimum of six fish of each colour and histology was conducted on an additional minimum of six fish of each colour at a fish disease diagnostic laboratory at the University of Florida in 2012 (OS2016 and PS2016) and 2017 (BS2017 and GS2017).

The reports did state that the findings were likely unrelated to the transgenic nature of the fish. Many species of ornamental fish have been reported to be susceptible to parasites (Florindo et al. 2017a,b; Iqbal et al. 2018; Trujillo-González et al. 2018). Parasites reported in Rainbow Sharks include metacercarial cysts of the trematode *Ascocotyle* sp. (Abdelhakiem et al. 2019), the dinoflagellate *Piscinoodinium* sp. (Wickins et al. 2011), and the ciliate *Ichthyophthirius multifiliis* (Kim et al. 2002). An external monogean trematode infection was also noted on albino Rainbow Sharks by Leggatt (2019) prior to their use in a temperature tolerance study. However, there are no reports of parasitic zoonoses associated with home aquarium exposure to *E. frenatum*.

In addition, no bacterial growth was observed after 48 hours (at 28°C) in brain and posterior kidney samples plated onto blood agar plates (TSA + 5% sheep's blood) for two (BS2017 and GS2017) of the notified lines. For OS2016, one of the six fish tested positive in the kidney at 24 hours with all other cultures negative at 48 hours. The bacterial species was not identifiable using the Biolog system and PCR identification was not pursued at request of the notifier. For PS2016, one of the six fish tested positive in the kidney for *Pseudomonas aeruginosa* at 24 hours with all other cultures negative at 48 hours. Antibiotic susceptibility was not assessed for either positive sample at request of the notifier. An in-house literature search found no reported cases of *P. aeruginosa* infection in humans attributed to exposure to tropical ornamental fish.

Allergenicity/Toxigenicity

In-house amino acid sequence analyses of all the expressed fluorescent proteins and the blue chromoprotein were done using the AllergenOnline Database (v22; 25 May, 2023) (http://www.allergenonline.org). Similar to previous analyses on these fluorescent proteins and the blue chromoprotein done on previously notified GloFish® lines, no matches with greater than 35% identity nor exact matches for 80 and 8 sliding window amino acid segments, respectively, were found for any of the fluorescent proteins or the blue chromoprotein. Similarly, results provided by the notifier from analyses using the Allermatch™ website (http://www.allermatch.org) found no matches for 80 amino acid sliding window alignments using the 35% cutoff or exact matches using segments 8 amino acids in length. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA plants (WHO/FAO 2009).

As seen with previously assessed green and purple GloFish® lines, analyses conducted for all the other reading frames found a positive result using the 80mer sliding window for a putative ORF in the 5'3' Frame 3 direction in the expression cassette sequences for GS2017 and in both the 3'5' Frames 1 and 3 directions for PS2016. The ORF in GS2017 was found to have 35.03% identity with a predicted collagen alpha-1(I) chain-like isoform X1 from the Barramundi (*Lates calcarifer*). However, the full-length alignment resulted in only 35.4% identity and there was a high E-value (expectation value) of 100. The ORFs in PS2016 were each found to have a 35.03% identity with serine protease from the fungus *Aspergillus niger*. Full length alignments resulted in 33.0% identities with high E-values of 1900 and 630. In the insert sequence of the expression cassette in BS2017, the same putative ORF was observed in 3'5' Frames 1 and 3 and was found to have 35.0% identity with the allergenic polypeptide Myr p I from the Jack jumper ant (*Myrmecia pilosula*). Full length alignment showed a 35.90% identity with an E-value of 120. Cross-reactivity typically requires the matches to be 40% identical over 80 amino acids with an E-value score of 1e-15 or less (Dr. Richard Goodman, University of Lincoln-Nebraska, personal communication). Thus, allergic cross reactivity is not likely for any of the four putative ORFs. In addition, Basic Local Alignment Search Tool (BLAST) analyses on the putative ORF amino acid sequences with BLASTP found no significant similarity to a known protein for GS2017 while approximately 57.2% identities to the same synthetic construct were found for the putative ORF sequences seen in BS2017 and PS2016. Analyses on the inserted nucleotide sequences for prediction of translation initiation sites using an online program (http://atgpr.dbcls.jp/) only found sites with a high reliability associated for the expected proteins. Therefore, these putative ORFs would most likely not result in expressed proteins in BS2017, GS2017 or PS2016.

BLAST analyses of all the inserted fluorescent protein and chromoprotein sequences do not indicate any homologies to sequences of potential toxins or allergens. No adverse effects were observed in male rats fed pure green fluorescent protein (GFP) or canola expressing GFP for 26 days (Richards et al. 2003). An in-house literature search found no reports of adverse effects attributed to any of the inserted proteins in humans. Furthermore, there is no evidence indicating any potential for BS2017, GS2017, OS2016, and PS2016, or E. frenatum to produce toxic or other hazardous materials that may accumulate in the environment or be consumed by humans or other organisms in the environment.

History of Use

Enforcement Discretion decisions by the U.S. Food and Drug Administration (USFDA) were received in 2017 (OS2016 and PS2016) and 2018 (BS2017 and GS2017) and these lines have since been commercially available in the U.S. The green fluorescent protein used in GS2017 has been used in other GloFish® lines since as early as 2006 while both proteins in BS2017 have been used since 2011. Fluorescent proteins used in PS2016 and OS2016 have been used since 2013 and 2017, respectively. Wild-type Rainbow Sharks have been sold worldwide as aquarium fish since the 1970s (Brand 2020).

HAZARD CHARACTERIZATION

The human health hazard potential of BS2017, GS2017, OS2016, and PS2016 is assessed to be **low** (Table 1) because:

1. BS2017, GS2017, OS2016, and PS2016 are genetically modified tropical fish containing transgene constructs at a single site of insertion (although alternate insert patterns may exist in the population) and that appear phenotypically stable based on line maintenance protocols;
2. The methods used to produce BS2017, GS2017, OS2016, and PS2016 do not raise any indirect human health concerns. While some of the source organisms from which the inserted genetic material was derived appear to produce toxins, there is no indication that any of the inserted genetic material or expressed proteins in these lines are associated with any toxicity or pathogenicity in humans;
3. While there are reported cases of zoonotic infections associated with tropical aquarium fish, particularly for immunocompromised individuals and children, there are no reported cases attributed to any of the commercially available lines of GloFish® or to wild-type Rainbow Sharks;
4. Sequence identities of the inserted transgenes do not match any known allergens or toxins. With the exception for OS2016, amino acid sequences of the fluorescent proteins and blue chromoprotein are identical to those used in previously assessed GloFish® lines. While analyses conducted on the other potential reading frames found potential matches in BS2017, GS2017, and PS2016, the results suggest there is little evidence for cross-reactivity; and
5. There is a history of safe use for the notified lines, as well as for the other commercially available lines of GloFish® and the wild-type Rainbow Shark has been safely used globally as an ornamental aquarium fish since the 1970s.

Table 1. Considerations for hazard severity (human health).

HAZARD	CONSIDERATIONS
High	• Effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal. • Prophylactic treatments are not available or are of limited benefit. • High potential for community level effects.
Medium	• Effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. • Some potential for community level effects
Low	• No effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals. • Effective prophylactic treatments are available. • No potential for community level effects.

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 2. Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organisms. Adequate information was also provided describing in good detail the methods used to genetically modify the wild-type *E. frenatum* including the sources of the genetic materials and the stability of the resulting genotypes and phenotypes. Sequence analyses of the inserted transgene constructs for the four notified lines did not match any known toxins or allergens and no reports were found of adverse effects attributed to the inserted proteins in humans.

While there were no reports of adverse human health effects directly associated with the notified organisms or the other commercially available lines of GloFish®, surrogate information from the literature on other ornamental fish appear to indicate the potential for transmission of human pathogens. However, this potential for infections is common to all ornamental aquarium fish and are not unique to Rainbow Sharks. The inserted fluorescent proteins (except for protein in OS2016) and blue chromoprotein have been used in other lines of GloFish® for several years and there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organisms, surrogate information from the literature on other ornamental aquarium fish and the lack of adverse effects supported by the history of safe use for other lines of GloFish®, the indirect human health hazard assessment of BS2017, GS2017, OS2016, and PS2016 is considered to be **low** with **low uncertainty**. The uncertainty is considered low because much of the information on human health effects are based on reports from other ornamental aquarium fish as there are a very limited number of studies in the scientific literature on *E. frenatum*. In addition, there is a limited history of safe use in the United States for the notified lines as they have only been in commercial production since 2017 (OS2016 and PS2016) or 2018 (BS2017 and GS2017) as well as the fact that there are no particular studies that have investigated human health effects associated with fluorescent transgenic ornamental fish.

Table 2. Categorization of uncertainty related indirect human health hazard.

Description	Uncertainty Ranking
There are many reports of human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e., low variability); OR The potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.	Negligible
There are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR There are no reports of human health effects and there are no effects related to the hazard reported for other mammals.	Low
There are some reports of human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent; OR There are reports of effects related to the hazard in other mammals but not in humans.	Moderate
Significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).	High

EXPOSURE ASSESSMENT

The generalized human exposure pathway for BS2017, GS2017, OS2016, and PS2016 is illustrated in Figure 1. The pathway describes the potential for human exposure through the intended use, other potential uses and environmental releases.

INTENDED USE

Import and distribution to retailers in Canada

Imported fish will enter Canada through various undisclosed points of entry. Broodstock are maintained using the same breeding protocol for all types of F2 fish that become the lines identified as BS2017, GS2017, OS2016, and PS2016. In the production locations in United States, the Division of Aquaculture of the Florida Department of Agriculture and Consumer Services regulates the production of the notified lines to ensure the use of best management practices and help protect the environment. The notifier intends to ship adult fish to distributors and eventually to pet stores in quantities ordered and held until sold to the public.

The notifier plans to market BS2017, GS2017, OS2016, and PS2016 fish in Canada using approximately 500 retail outlets based on market size relative to United States. The exact number and locations where the notified organisms will be available for sale are not currently known. As ornamental fish intended for sale to the public, it is anticipated that they will be confined inside aquaria in homes and retail outlets. For the intended use, human exposure could happen during distribution involving the transportation of fish by the importer as well as during storage, handling and sale by the retailer. Based on a survey of store owners in Montreal, Quebec, fish are either kept and put on sale by retailers until sold or returned to the distributor and are less likely to be released into the environment by retailers (Gertzen et al. 2008). For BS2017, GS2017, OS2016 and PS2016, it is unclear if the notifier allows for retailers to return unsold fish to distributors. Since retailers are not expected to be the final users of BS2017, GS2017, OS2016, and PS2016, human exposure during importation and distribution to retailers is expected to be largely occupational.

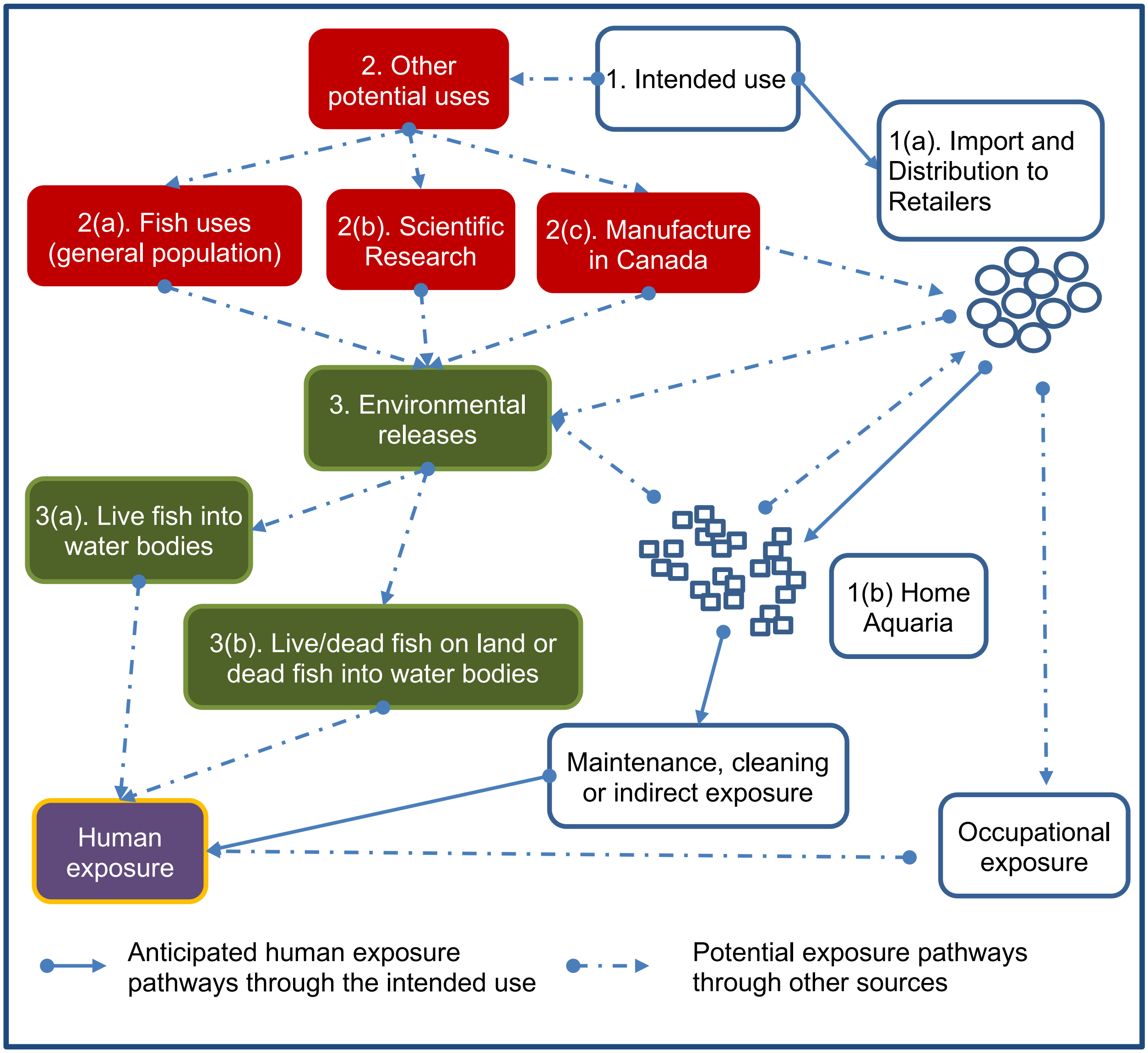

Figure 1. Generalized human exposure pathways for BS2017, GS2017, OS2016, and PS2016.

Introduction in Canada as ornamental fish in home aquaria

Human exposure by home aquarists that purchase the notified lines directly from retailers or receive them from other aquarists will most likely occur through contact with the notified fish during maintenance activities such as water changes and tank cleanings. Information on stocking rate per household and the number of households planning to purchase the notified lines would be helpful in estimating human exposure to BS2017, GS2017, OS2016 and PS2016 through the intended use, as ornamental fish in home aquaria. In absence of this information, an indication of the number of GloFish® sharks per household could be estimated based on the recommended stocking rate and the proportion of Canadians expected to purchase the notified lines.

Because of aggressive behaviour, the notifier recommends a minimum of 110 litres (29-gallon) aquarium for a single shark and 208 or 227 L (55 or 60 gallon) tanks for more than one shark. Similar aquaria sizes are recommended for wild type Rainbow Shark (Brand 2020). The need for a large tank and the aggressive behaviour would most likely limit the number of GloFish® sharks to one per household.

Furthermore, there are about 16 million households in Canada based on the 2021 census (Statistics Canada 2021). A 2009 survey estimated that approximately 12% of Canadian households owned fish (Perrin 2009) and another survey (Marson et al. 2009) reported approximately 16% of respondents (66 out of 418) having species of minnow Sharks in their aquaria. Assuming the same proportion of Canadians practice some form of fishkeeping at present, approximately 307,200 households could come in contact with GloFish® fluorescent *E. frenatum* lines BS2017, GS2017, OS2016, and PS2016 (i.e., 16% owning minnow Sharks of 12% having fish as pets of the 16 million households).

The recommended temperatures for home aquaria established for *E. frenatum* is between 24°C and 27°C (Brand 2020). As with other ornamental fish, these temperatures and conditions in aquariums also favour the growth of opportunistic pathogens like *M. marinum* (Kent et al. 2006; Mutoji and Ennis 2012; Gauthier 2015) or parasites like *Cryptosporidium* species (Ryan et al. 2015; Golomazou and Karanis 2020). We have no knowledge of the health status of people that may be exposed to BS2017, GS2017, OS2016, and PS2016. It is reasonable to assume that the households intending to purchase the notified lines could include immunocompromised individuals, children and those with underlying medical conditions.

OTHER POTENTIAL USES

Fish uses for the general population

For the notified lines (BS2017, GS2017, OS2016 and PS2016), some potential uses have been identified for the general population in addition to the intended use as ornamental fish in home aquaria. Considering the similarity to other members of the family cyprinidae (carps and minnows) that are used as baitfish in Canada (Kerr et al. 2005; Cudmore and Mandrak 2022), it is plausible for the notified lines to be used as bait fish. They may also be grown in ornamental outdoor fishponds and even consumed by humans as part of the live food fish industry. These uses are some of the pathways identified by Kerr et al. (2005) as being the potential pathways of introduction of aquatic species in Ontario. However, the notifier argues that BS2017, GS2017, OS2016 and PS2016 are not suitable for use in outdoor ponds, or for human consumption, or as bait fish. Year-round growth in outdoor ponds may be difficult in Canada for the notified lines due to their lack of tolerance to low temperatures and cost relative to normal bait fish can be prohibitive for use of the notified lines as bait.

Scientific research

Each year, about 500,000 fish are used for research and education in Canada (Grey and Vincent 2006) with the number released for 2020 being 594,770 (CCAC 2021). In research, Rainbow Sharks have been used for the study of induction spawning (Elakkanai et al. 2017; Sipos et al. 2020) and the study of telocytes as part of cell biology, immunology and stem cells

(Abd-Elhafeez et al. 2020). Since fish are commonly used in the detection of pollutants in the environment (Evans et al. 2005), use of the notified fish as an environmental sentinel cannot be excluded from the list of potential uses.

Manufacture in Canada

Manufacture of the notified organisms is not anticipated to occur in Canada as BS2017, GS2017, OS2016, and PS2016 are only produced in Florida. In the production locations in the United States, outdoor freshwater ponds of 10-20 cubic meters volume that can house up to 10,000-15,000 fish are used to grow most of the fish. During summer, the ponds are either open or covered with bird netting to reduce fish loss due to predation and partially covered with dark plastic to provide shade. In winter, the ponds are covered with transparent plastic to prevent heat loss. When ready to spawn or to be sold, the fish are collected from the ponds and held indoors in vats holding 700-900 liters of freshwater that accommodate over 1000 fish. Because of low tolerance of Rainbow Sharks to cold temperatures, outdoor ponds are assumed to be not practical in Canada. However, should manufacture occur in Canada using indoor tanks or other means, no additional potential for exposure is foreseen that is different from any other typical aquarium fish.

Conclusion on potential uses for general population

While not completely ruling out other potential uses, no significant exposure is anticipated should the notified lines be produced in Canada (indoor or outdoor), used as bait fish, as food, as environmental sentinels, or in research.

ENVIRONMENTAL RELEASES AND FATE IN THE ENVIRONMENT

According to the notifier, the intended use of BS2017, GS2017, OS2016, and PS2016 is not for environmental release but rather for use in home aquaria. However, accidental, unintended or deliberate environmental releases into water bodies or other media (e.g. land, landfill, etc.) cannot be ruled out since there are reports of releases of ornamental fish from home aquaria due to a variety of reasons (Duggan et al. 2006; Gertzen et al. 2008; Chan et al. 2019). Ornamental fish are often deliberately released into the environment as a 'humane' method of disposal of unwanted pets or when they develop undesired characteristics (Chan et al. 2019). Aquarists could potentially release unwanted aquarium fish into the environment when they become bored with the fish or when fish become aggressive, sick, large in size, or have high reproductive output (Gertzen et al. 2008; Chan et al. 2019). Ornamental fish could also be released into the environment through disposal of aquarium wastes on lawns, gardens, outdoor ponds or stormwater drains (Duggan et al. 2006). There are no records of deliberate or accidental release of Rainbow Sharks into the environment but this species is known to be aggressive towards same or different species leading to injuries to other fish (Mohsin and Mondal 2013). It is also reported to have a tendency to jump out of fish tanks (Brown et al. 2008).

Releases of live fish into water bodies

In an event of environmental releases of live BS2017, GS2017, OS2016, and PS2016 into waterways in Canada, future establishment will depend on environmental conditions at the point of release and the ability of the released fish to survive, grow, reproduce, disperse and establish self-sustaining populations (Duggan et al. 2006; Strecker et al. 2011; Leggatt et al. 2018; Leggatt 2019). Temperature tolerance is a key criterion for determining the ability of aquarium fish to survive, establish and overwinter in the Great Lakes and in Canadian waters as a whole (Rixon et al. 2005; DFO 2018; Leggatt et al. 2018; Leggatt 2019).

Results from temperature tolerance tests found all transgenic genotypes showed slightly higher sensitivity to cold-water temperatures compared to their non-transgenic siblings but still within the lethal water temperature ranges for *E. frenatum*. However, the temperature tolerance of OS2016 was not significantly different compared to that of the wild-type (non-fluorescent) siblings. Leggatt (2019) reported similar temperature tolerance limits (LD_{50} = 10.7 ± 0.1 °C) in wild-type albino Rainbow Sharks.

Even the most cold tolerant *E. frenatum* lost equilibrium in temperatures several degrees above typical winter water temperatures in Canada (4°C or less) and temperatures in the warmest recorded lakes of 6°C or less in winter (Leggatt et al. 2018; Leggatt 2019). It is therefore less likely for BS2017, GS2017, OS2016 and PS2016 lines to survive and disperse in typical winter water temperatures in Canada. There is however a potential for persistence of *E. frenatum* in isolated warm pockets of water (e.g. hot springs, thermal effluent from industrial sites), although it is not known if the biotic and abiotic requirements for Rainbow Shark survival and reproduction would be met in these locations (Leggatt 2019). Della Venezia et al. (2018) examined the potential establishment of freshwater ornamental fish in North America under a climate change scenario forecast for the year 2050. According to the authors, while the model forecasted a two-fold average invasion risk in Quebec, the establishment risk remained extremely low as the minimum temperature for the coldest month would not likely be high enough in 2050 to make Quebec suitable to potential invasions from species currently in the ornamental trade. Tuckett et al. (2017) reported one escaped Rainbow Shark captured less than 500 m from an aquaculture facility in Florida with none captured beyond 500 m. This suggests that even in warmer climates that any released Rainbow Sharks would not be expected to survive and disperse (Leggatt 2019). Therefore, the likelihood of BS2017, GS2017, OS2016, and PS2016 establishing self-sustaining populations in Canada is very low due to their inability to survive water temperatures lower than 10°C based on temperature tolerance studies. Therefore, the likelihood of human exposure to the notified organisms in the environment is low.

Release of live or dead fish on land or dead fish into water bodies

In the event a fish dies before sale to or while in the care of a home aquarist, the notifier suggests a disposal procedure similar to all other domestic waste and there are no special handling or disposal procedures required. The notifier has indicated that no specific procedures or treatments are required for disposal of the notified organisms (BS2017, GS2017, OS2016, and PS2016) compared to the wild-type species as the only difference (for each line) is the addition of a fluorescent protein (and chromoprotein in BS2017) derived from species of coral, jellyfish, or sea anemones. The notifier recommends that individuals that no longer wish to maintain the organisms after purchase either return them to the retailer, give them to another aquarium hobbyist, or humanely euthanize them. Additionally, sale of these lines can be halted at any time if it is determined necessary to terminate the introduction of BS2017, GS2017, OS2016, and PS2016 in Canada. It can be assumed that live or dead fish released onto garden lawns, fields or into landfills or dead fish released into water bodies would not lead to survival and establishment in the environment. Therefore, any live or dead BS2017, GS2017, OS2016 and PS2016 released into the environment, as well as their respective fluorescent and chromo-proteins are expected to biodegrade normally and not accumulate or be involved in biogeochemical cycling in a manner different from other living organisms.

EXPOSURE CHARACTERIZATION

Risks from workplace exposure to the notified lines are not considered in this assessment[1].

The human exposure potential of BS2017, GS2017, OS2016, and PS2016 is assessed to be **low to medium** (Table 3) because:

1. The primary sources of human exposures would stem from the proposed import of adult fish for the four lines (BS2017, GS2017, OS2016, and PS2016) through unidentified points of entry in Canada and distribution through about 500 retail outlets;
2. The sole intended use of BS2017, GS2017, OS2016, and PS2016 is as ornamental aquarium fish, thus limiting potential exposure primarily to those possessing a home aquarium;
3. Like other aquarium fish, human exposure may include immunosuppressed individuals, children, those with underlying medical conditions or other vulnerable individuals;
4. Typical human exposure to live or dead fish in the home is most often related to maintenance activities such as tank cleanings and water changes. Low winter water temperatures in Canadian waters and low cold tolerance of notified fish limits human exposure through the environment;
5. Any release of live or dead BS2017, GS2017, OS2016, or PS2016 on garden lawns, fields or into landfills or into water bodies is not expected to result in survival and establishment in the environment. Should there be such releases, both fish and their respective fluorescent and chromo- proteins are expected degrade normally and not accumulate or result in human exposure; and
6. No significant increase in human exposure is expected from other potential uses of BS2017, GS2017, OS2016, and PS2016 such as for research, as bait or as environmental sentinels.

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Uncertainty ranking associated with the information used to assess indirect human health exposure for BS2017, GS2017, OS2016, and PS2016 is presented in Table 4. As indicated, the notified organisms will not be manufactured in Canada and the source of exposure will be restricted to the import of adult fish for the four lines. In the environment, empirical data supports the conclusion that the survival of these fish is expected to be limited by their poor tolerance to temperatures below 10°C. However, this does not preclude the potential for human exposure (general public and vulnerable individuals [i.e., immunocompromised, children, medical conditions, etc.]) in Canada through home aquaria mainly from maintenance and cleaning activities. This exposure assessment is limited by the lack of information on actual number of notified organisms to be imported in subsequent years and poor survey data on household ownership of ornamental fish. It is therefore difficult to gauge public uptake and popularity

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the Hazardous Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

beyond the import number in the first year. Furthermore, household surveys looking into aquarium fish ownership in Canada based on reports from more than 10 years ago (Duggan et al. 2006; Gertzen et al. 2008; Marson et al. 2009; Perrin 2009). These reports are not specific to BS2017, GS2017, OS2016, or PS2016 and do not investigate factors influencing human exposure to aquarium fish. Therefore, because of limited information on the specific exposure scenarios in the Canadian market, the human exposure to the notified organisms is considered low to medium with moderate uncertainty.

Table 3. Exposure considerations (human health).

Exposure	Considerations
High	• The release quantity, duration and/or frequency are high. • The organism is likely to survive, persist, disperse proliferate and become established in the environment. • Dispersal or transport to other environmental compartments is likely. • The nature of release makes it likely that susceptible populations or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem. • In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible organisms.
Medium	• The organism is released into the environment, but quantity, duration and/or frequency of release is moderate. • The organism may persist in the environment, but in low numbers. • The potential for dispersal/transport is limited. • The nature of release is such that some susceptible populations may be exposed. • In relation to exposed humans, routes of exposure are not expected to favour toxic, zoonotic or other adverse effects.
Low	• The organism is used in containment (no intentional release). • The nature of release and/or the biology of the organism are expected to contain the organism such that susceptible populations or ecosystems are not exposed. • Low quantity, duration and frequency of release of organisms that are not expected to survive, persist, disperse or proliferate in the environment where released.

Table 4. Uncertainty ranking associated with the indirect human health exposure.

Available Information	Uncertainty Ranking
High quality data on the organism, the sources of human exposure and the factors influencing human exposure to the organism. Evidence of low variability.	Negligible
High quality data on relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism or valid surrogate. Evidence of variability.	Low
Limited data on the organism, relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism.	Moderate
Significant knowledge gaps. Significant reliance on expert opinion.	High

RISK CHARACTERIZATION

NOTIFIED USE

In this assessment, risk is characterized according to the paradigm: Risk $\propto$ Hazard x Exposure. The two components (“hazard” and “exposure”) are considered embedded in the definition of “toxic” under section 64 of CEPA 1999 and hence, there is no risk in absence of either. The risk assessment conclusion is based on the hazard, and on what we can predict about exposure from the notified use.

BS2017, GS2017, OS2016, and PS2016 are genetically modified lines of diploid, hemizygous or homozygous, Rainbow Sharks containing fluorescent protein (and blue chromoprotein in BS2017) genetic constructs derived from species of sea anemones, jellyfish, or soft corals which makes them appear blue (BS2017), green (GS2017), orange (OS2016), and purple (PS2016) under ambient light, including sunlight. All four lines were derived from a line of domesticated albino Rainbow Shark.

The notified organisms will be marketed throughout Canada for use as ornamental fish in home aquaria.

Although there are reported cases of zoonotic infections from exposure to aquarium fish, wild type Rainbow Sharks are popular in home aquaria with a long history of safe use having been sold worldwide as aquarium fish since the 1970s (Brand 2020). Enforcement Discretion decisions by the USFDA were received in 2017 for OS2016 and PS2016 and in 2018 for BS2017 and GS2017 and all have been commercially available since in the United States. With the exception of the fluorescent protein found in OS2016, the fluorescent proteins used in the other three notified lines have been used in other GloFish® lines that are now commercially available in Canada. There are no reported adverse human health effects associated with wild type Rainbow Sharks in general, the inserted fluorescent protein or chromoprotein genes and the methods used to modify the notified lines leading to a conclusion that the notified lines do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of *E. frenatum* BS2017, GS2017, OS2016, or PS2016 as ornamental aquarium fish is assessed to be low.

OTHER POTENTIAL USES

Other uses identified include the use of the notified organisms for research purposes, as bait fish and for pollution detection (environmental sentinel). Regardless of the use, the available information does not indicate a potential human health implication. No additional risks to human health are foreseen that are different from those of any other typical aquarium fish.

RISK ASSESSMENT CONCLUSION

There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from the use of BS2017, GS2017, OS2016, or PS2016 as ornamental aquarium fish or any other potential uses. This risk to human health associated with BS2017, GS2017, OS2016, or PS2016 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

REFERENCES CITED

Abd-Elhafeez, H.H., Abdo, W., Kamal, B.M., and Soliman, S.A. 2020. Fish teleocytes and their relation to rodlet cells in ruby-red-fin shark (rainbow shark) *Elpazeorhynchos frenatum* (Teleostei: Cyprinidae). Sci. Rep. 18907.

Abdelhakiem, A., Soliman, S.A., Adb-Elhafeez, H.H., Abdel-Hafez, E., and Zaki, R.S. 2019. Occurrence of metacercarial cyst of *Ascocotyle* (*Ascocotyle* sp.) in the gills of ruby-red-fin shark (rainbow shark) *Epalzeorhynchos frenatum* (Teleostei:Cyprinidae): light microscopic study. EC Clin. Exp. Anat. 2(7):296-304.

Aqua-fish. 2014. Rainbow shark – care, behaviour, feeding & forum. URL: https://en.aqua-fish.net/articles/advice-keeping-rainbow-sharks-focusing-temperament-tank-setup. Accessed November 10, 2023.

Baiano, J.C.F., and Barnes, A.C. 2009. Towards control of *Streptococcus iniae*. Emerg. Infect. Dis. 15:1891-1896.

Boylan, S. 2011. Zoonoses associated with fish. Vet. Clin. Exot. Anim. 14:427-438.

Brand, R. 2020. The complete rainbow shark care guide (*Epalzeorhynchos frenatum*). URL: https://aquariumtidings.com/rainbow-shark-care-guide/. Accessed October 3, 2023.

Brown, C.L., Cole, B., Farfan, C., and Tamaru, C.S. 2008. Out-of-season spawning of the rainbow shark, *Epalzeorhynchos frenatus*: freshwater hatchery technology with marine potential. Marine Ornamental Species: Collection, Culture & Conservation. pp. 255-262.

Cameselle-Martínez, D., Hernández, J., Francès, A., Montenegro, T., Canas, F., and Borrego, L. 2007. Sporotrichoid cutaneous infection by *Mycobacterium haemophilum* in an AIDS patient. Actas Dermo-Sifiliográficas. 98(3):188-193.

Cardoso, P.H.M., Moreno, A.M., Moreno, L.Z., de Oliveira, C.H., de Assis Baroni, F., de Lucca Maganha, S.R., de Sousa, R.L.M., and de Carvalho Balian, S. 2019. Infectious diseases in aquarium ornamental pet fish: Prevention and control measures. Braz. J. Res. Anim. Sci. 56(2):e151697.

Cardoso, P.H.M., Moreno, L.Z., de Oliveira, C.H., Gomes, V.T.M., Silva, A.P.S., Barbosa, M.R.F., Sato, M.I.Z., Balian, S.C., and Moreno, A.M. 2021. Main bacterial species causing clinical disease in ornamental freshwater fish in Brazil. Folia Microbiologica. 66:231-239.

CCAC. 2021. CCAC animal data report 2020. URL: https://ccac.ca/Documents/AUD/CCAC_Animal_Data_Report_2020.pdf. Accessed November 17, 2023.

CDC. 2023. Healthy pets, healthy people. Centers for Disease Control and Prevention, URL: https://www.cdc.gov/healthypets/pets/fish.html. Accessed November 17, 2023.

Chan, F.T., Beatty, S.J., Gilles Jr., A.S., Hill, J.E., Kozic, S., Luo, D., Morgan, D.L., Pavia Jr., R.T.B., Therriault, T.W., Verreycken, H., Vilizzi, L., Wei, H., Yeo, D.C.J., Zeng, Y., Zięba, G., and Copp, G.H. 2019. Leaving the fish bowl: the ornamental trade as a global vector for freshwater invasions. Aquatic Ecosystem Health & Management. 22(4)417-439.

Cremonesini, D., and Thomson, A. 2008. Lung colonization with *Aeromonas hydrophila* in cystic fibrosis believed to have come from a tropical fish tank. J. R. Soc. Med. 101:S44-S45.

Cudmore, B., and Mandrak, N.E. 2022. The baitfish primer: a guide to identifying and protecting Ontario's baitfishes. 2022 version. DFO/2022-2000. Catalogue number: Fs134-7/2023E.

Delghandi, M.R., El-Matbouli, M., and Menanteau-Ledouble, S. 2020. Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: A review. Microorganisms. 8(9)1368:1-18.

Della Venezia, L., Samson, J., and Leung, B. 2018. The rich get richer: invasion risk across North America from the aquarium pathway under climate change. Diversity and Distributions. 24:285-296.

DFO. 2018. Environmental and indirect human health risk assessment of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra (*Gymnocorymbus ternetzi*): a transgenic ornamental fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2018/027.

Dinç, G., Doğanay, M., and Izgür, M. 2015. Important bacterial infections transmitted to humans from pet animals. Turk Hijyen ve Deneysel Biyoloji Dergisi. 72(2):163-174.

Duggan, I. C., Rixon, C. A., and MacIsaac, H. J. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological invasions. 8(2):377-382.

Dunn, J.R., Behravesh, C.B., and Angulo, F.J. 2015. Diseases transmitted by domestic livestock: Perils of the petting zoo. Microbiol. Spectrum. 3(6):IOL5-0017-2015.

Edmond, A. 2022. Rainbow shark care guide: tank setup, diet, breeding, and more. URL: https://theaquariumguide.com/articles/rainbow-shark. Accessed November 10, 2023.

Elakkanai, P., Francis, T., Ahilan, B., Jawahar, P., Padmavathy, P., and Subburaj, A. 2017. Effect of synthetic kisspeptin-10, hCG and GnRH on induced breeding of *Epalzeorhynchos frenatum*. J. Exp. Zool. India. 20(2):923-927.

Emmerich, K., Kolb-Mäurer, A., and Goebeler, M. 2019. Cutaneous infections due to non-tuberculous mycobacteria. Aktuelle Dermatologie. 45(1-2):47-51.

Evans, M. S., Muir, D., Lockhart, W. L., Stern, G., Ryan, M., & Roach, P. 2005. Persistent organic pollutants and metals in the freshwater biota of the Canadian Subarctic and Arctic: an overview. Science of the Total Environment, 351, 94-147.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Gonçalves, E.L.T., Cardoso, L., and Martins, M.L. 2017a. Protozoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):948-956.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Figueredo, A.B., Gonçalves, E.L.T., Cardoso, L., da Costa Marchiori, N., da Costa Assis, G., and Martins, M.L. 2017b. Metazoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):992-998.

Franco-Paredes, C., Chastain, D.B., Allen, L., and Henao-Martínez, A.F. 2018. Overview of cutaneous mycobacterial infections. Curr. Trop. Med. Rep. 5(4):228-232.

Franco-Paredes, C., Marcos, L.A., Henao-Martínez, A.F., Rodríguez-Morales, A.J., Villamil-Gómez, W.E., Gotuzzo, E., and Bonifaz, A. 2019. Cutaneous mycobacterial infections. Clin. Microbiol. Rev. 32(1):e00069-18.

Gaulin, C., Vincent, C., and Ismaïl, J. 2005. Sporadic infections of *Salmonella* paratyphi B, var. Java associated with fish tanks. Can. J. Public Health 96(6):471-474.

Gauthier, D.T. 2015. Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. Vet. J. 203:27-35.

Gertzen, E., Familiar, O., and Leung, B. 2008. Quantifying invasion pathways: fish introductions from the aquarium trade. Can. J. Fish. Aquat. Sci. 65(7):1265-1273.

Glajzner, P., Szewczyk, E.M., and Szemraj, M. 2021. Pathogenicity and drug resistance of animal streptococci responsible for human infections. J. Med. Microbiol. 70:001339.

Golomazou, E., and Karanis, P. 2020. *Cryptosporidium* species in fish: An update. In Environmental Sciences Proceedings (Vol. 2, No. 1, p. 13). Multidisciplinary Digital Publishing Institute.

Grey, M., and Vincent, A.C.J. 2006. Extent and possible conservation implications of fish use for research, testing and education in North America. Aquatic Conserv: Mar. Freshw. Ecosyst. 16:569-578.

Guz, L., and Puk, K. 2022. Antibiotic susceptibility of mycobacteria isolated from ornamental fish. J. Vet. Res. 66:69-76.

Haenen, O.L.M., Evans, J.J., and Berthe, F. 2013. Bacterial infections from aquatic species: Potential for and prevention of contact zoonoses. Rev. Sci. Tech. Off. Int. Epiz. 32:497-507.

Haenen, O., Karunasagar, I., Manfrin, A., Zrncic, S., Lavilla-Pitogo, C., Lawrence, M., Hanson, L., Subasinghe, R., Bondad-Reantaso, M.G., and Karunasagar, I. 2020. Contact-zoonotic bacteria of warmwater ornamental and cultured fish. Asian Fish. Sci. 33(S1):39-45.

Haenen, O.L.M., Dong, H.T., Hoai, T.D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S.L., Zadoks, R., Kiermeier, A., Wang, B., Gamarro, E.G., Takeuchi, M., Azmai, M.N.A., Fouz, B., Pakingking Jr., R., Wei, Z.W., and Bondad-Reantaso, M.G. 2023. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev. Aquac. 15(Suppl. 1):154-185.

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A., Marei, A., and Sitohy, M. 2018. *Mycobacterium marinum* infection in fish and man: epidemiology, pathophysiology and management; a review. Veterinary Quarterly. 38(1):35-46.

Herzog, P. 2023. Rainbow shark: full care guide (with setup, mates & diet). URL: https://fishtankadvisor.com/rainbow-shark/. Accessed October 27, 2023.

Hossain, S., De Silva, B.C.J., Dahanayake, P.S., and Heo G.-J. 2018. Characterization of virulence properties and multi-drug resistance profiles in motile *Aeromonas* spp. isolated from zebrafish (*Danio rerio*). Lett. Appl. Microbiol. 67:598-605.

Iqbal, Z., Ansar, F., and e-Huma, Z. 2018. Risk of importing zoonotic diseases through infected ornamental fish. Punjab University Journal of Zoology. 33(2):211-215.

Kamijo, F., Uhara, H., Kubo, H., Nakanaga, K., Hoshino, Y., Ishii, N., and Okuyama, R. 2012. A case of mycobacterial skin disease caused by *Mycobacterium peregrinum*, and a review of cutaneous infection. Case Rep. Dermatol. 4(1):76-79.

Kent, M. L., Watral, V., Wu, M., and Bermudez, L. E. 2006. *In vivo* and *in vitro* growth of *Mycobacterium marinum* at homoeothermic temperatures. FEMS Microbiology Letters 257(1):69-75.

Kerr, S.J., Brousseau, C.S., and Muschett, M. 2005. Invasive aquatic species in Ontario: a review and analysis of potential pathways for introduction. Fisheries. 30(7):21-30.

Kim, J-H., Hayward, C.J., Joh, S-J., and Heo, G-J. 2002. Parasitic infections in live freshwater tropical fishes imported to Korea. Dis. Aquat. Org. 52:169-173.

Lazado, C.C., and Zilberg, D. 2018. Pathogenic characteristics of *Aeromonas veronii* isolated from the liver of a diseased guppy (*Poecilia reticulata*). Lett. Appl. Microbiol. 67:476-483.

Leggatt, R.A., Dhillon, R.S., Mimeault, C., Johnson, N., Richards, J.G., and Devlin, R.H. 2018. Low-temperature tolerances of tropical fish with potential transgenic applications in relation to winter water temperatures in Canada. Can. J. Zool. 96(3):253-260.

Leggatt, R. A. 2019. Cold temperature tolerance of albino rainbow shark (*Epalzeorhynchos frenatum*), a tropical fish with transgenic application in the ornamental aquarium trade. Canadian Journal of Zoology, 97(4), 376-378.

Leighton, M. 2023. How to care for rainbow sharks: a complete fact sheet, breeding, behavior, and care guide. URL: https://www.vivofish.com/rainbow-sharks/. Accessed October 27, 2023.

Li, J., Chong, A.H., O'Keefe, R., and Johnson, P.D.R. 2014. The fish tank strikes again: Metachronous nontuberculous mycobacterial skin infection in an immunosuppressed host. Austral. J. Dermatol. 55:e77-e79.

Lowry, T., and Smith, S.A. 2007. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. J. Am. Vet. Med. Assoc. 231:876-880.

Marson, D., Cudmore, B., Drake, D.A.R., and Mandrak, N.E. 2009. Summary of a survey of aquarium owners in Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 2905: iv + 20 p.

Mohsin, A.B.M., and Mondal, R.C. 2013. Behaviour of 14 exotic ornamental fishes of Bangladesh under starved condition in aquaria. J. Fish. 1(1):48-52.

Musto, J., Kirk, M., Lightfoot, D., Combs, B.G., and Mwanri, L. 2006. Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003-04. CDI 30:222-227.

Mutoji, K.N., and Ennis, D.G. 2012. Expression of common fluorescent reporters may modulate virulence for *Mycobacterium marinum*: dramatic attenuation results from GFP over-expression. Comp. Biochem. Physiol. C 155:39-48.

Narendrakumar, L., Sudhagar, A., Preena, P.G., Nithianantham, S.R., Mohandas, S.P., and Swaminathan, T.R. 2022. Detection of *Mycobacterium marinum* and multidrug-resistant bacteria in a chronic progressive disease outbreak among Siamese fighting fish (*Betta splendens*) in India. Biologia. 77(9):2725-2733.

Ocean Info. 2023. Rainbow shark. URL: https://oceaninfo.com/animals/rainbow-shark/. Accessed October 10, 2023.

Page, A. 2021. GloFish shark care guide. URL: https://www.aquariadise.com/glofish-shark/. Accessed October 27, 2023.

Pate, M., Ovca, A., Jenčič, V., Žolnir-Dovč, M., and Ocepek, M. 2019. Mycobacteria in aquarium fish: Are fish handlers aware of their zoonotic potential. Slov. Vet. Res. 56(2):53-58.

Perrin, T. 2009. The business of urban animals survey: the facts and statistics on companion animals in Canada. The Canadian Veterinary Journal. 50(1):48.

Phillips Savage, A.C.N., Blake, L., Suepaul, R., McHuch, O'S., Rodgers, R., Thomas, C., Oura, C., and Soto, E. 2022. Piscine mycobacteriosis in the ornamental fish trade in Trinidad and Tobago. J. Fish Dis. 45(4):547-560.

Ponpornpisit, A., Endo, M., and Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. Fish. Sci. 66:1026-1031.

Puk, K., and Guz, L. 2020. Occurrence of *Mycobacterium* spp. in ornamental fish. Ann. Agric. Environ. Med. 27(4):535-539.

Purser, P. 2020. Into the deep: a survey of freshwater sharks. URL: https://www.tfhmagazine.com/articles/freshwater/into-the-deep-a-survey-of-freshwater-sharks-full-article#:~:text=Although%20these%20two%20sharks%20look,in%20the%20red%2Dtail%20shark. Accessed October 27, 2023.

Richards, H.A., Han, C-T., Hopkins, R.G., Failla, M.L., Ward, W.W., and Stewart, C.N. Jr. 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. J. Nutr. 133(6):1909-1912.

Rixon, C.A., Duggan, I.C., Bergeron, N.M., Ricciardi, A., and MacIsaac, H.J. 2005. Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great Lakes. Biodiv. Conserv. 14:1365-1381.

Roberts, H.E., Palmeiro, B., and Weber, E.S. 2009. Bacterial and parasitic diseases of pet fish. Veterinary Clinics of North America: Exotic Animal Practice. 12(3):609-638.

Russo, R., Mitchell, H., and Yanong, R.P.E. 2006. Characterization of *Streptococcus iniae* isolated from ornamental cyprinid fishes and development of challenge models. Aquacul. 256:105-110.

Ryan, U., Paparini, A., Tong, K., Yang, R., Gibson-Kueh, S., O'Hara, A., Lymbery, A. and Xiao, L. 2015. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata*). Experimental Parasitology, 150, pp.31-35.

Seriously Fish 2023. *Epalzeorhynchos frenatum* (Fowler, 1934). Rainbow Shark. URL: https://www.seriouslyfish.com/species/epalzeorhynchos-frenatum/. Accessed October 26, 2023.

Shireman, J.V. and Gildea, J.A. 1989. Induced spawning of rainbow sharks (*Labeo erythrurus*) and redtail black sharks (*L. bicolor*). The Progressive Fish-Culturist. 51(2):104-108.

Sipos, M.J., Lipscomb, T.N., Wood, A.L., Ramee, S.W., Watson, C.A., and DiMaggio, M.A. 2020. Evaluation of cGnRH IIa for induction of spawning of two ornamental *Epalzeorhynchos* species. Aquacult. Res. 51:232-241.

Statistics Canada. 2021. (table). Census Profile. 2021 Census of Population. Statistics Canada Catalogue no. 98-316-X2016001. Ottawa. Released April 27, 2022. https://www12.statcan.gc.ca/census-recensement/2021/dp-pd/prof/index.cfm?Lang=E. Accessed November 10, 2023.

Strecker, A.L., Campbell, P.M., and Olden, J.D. 2011. The aquarium trade as an invasion pathway in the Pacific Northwest. Fisheries. 36(2):74-85.

Tropical Fish Keeping. 2016. Red fin shark (*Epalzeorhynchos munense*). URL: https://tropical-fish-keeping.com/red-fin-shark-epalzeorhynchos-munense.html. Accessed October 27, 2023.

Trujillo-González-A., Becker, J.A., and Hutson, K.S. 2018. Parasite dispersal from the ornamental goldfish trade. Adv. Parasit. 100:239-281.

Tuckett, Q.M., Ritch, J.L., Lawson, K.M., and Hill, J.E. 2017. Landscape-scale survey of non-native fishes near ornamental aquaculture facilities in Florida, USA. Biol. Invasions. 19:223-237.

Weir, M., Rajić, A., Dutil, L., Cernicchario, N., Uhland, F.C., Mercier, B., and Tuševljak, N. 2012. Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: A systematic review of the existing research and survey of aquaculture-allied professionals. Epidemiol. Infect. 140:192-206.

Whitfield, Y., and Smith, A. 2014. Household pets and zoonoses. Environ. Health Rev. 57(2):41-49.

WHO/FAO. 2009. Foods derived from modern biotechnology, 2nd edition. Rome, Italy: World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO), Codex Alimentarius. Available at: http://www.fao.org/3/a-a1554e.pdf.

Wickins, S.C., Dennis, M.M., Landos, M., Šlapeta, J., and Whittington, R.J. 2011. Histopathological survey of lesions and infections affecting sick ornamental fish in pet shops in New South Wales, Australia. Dis. Aquat. Org. 94:143-152.

Woods, R. 2022. Rainbow shark care guide & species profile. URL: https://www.fishkeepingworld.com/rainbow-shark/. Accessed October 27, 2023.

Yanar, M., Erdoğan, E. and Kumlu, M. 2019. Thermal tolerance of thirteen popular ornamental fish Species. Aquaculture, 501, pp.382-386.

Yang, A. 2022. Rainbow shark 101: care guide, tank size, food & tank mates. URL: https://www.aquariumsource.com/rainbow-shark/. Accessed October 27, 2023.

Yanong, R.P.E., Russo, R., and Mitchell, H. 2007. A mult-pronged approach to streptococcosis in rainbow sharks (*Epalzeorhynchos frenatum*) and redtail black sharks (*E. bicolor*). URL: https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11257&catId=32371&id=3865305. Accessed October 11, 2023.

Ziarati, M., Zorriehzahra, M.J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., Bin Emran, T., Dhama, K., Chaicumpa, W., and Shamsi, S. 2022. Zoonotic diseases of fish and their prevention and control. Vet. Quart. 42(1):95-118.